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Prevalence study of drugs and new psychoactive substances in hair of ketamine consumers using a methanolic direct extraction prior to high-resolution mass spectrometry☆

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ABSTRACT

Few studies have reported the prevalence or incidence about the consumption of new psychoactive substances (NPS). The hair analysis can be useful for this purpose. At the present, ketamine is the most consumed arylcyclohexylamine associated to young consumers and polyconsumption profiles. For this reason, ketamine consumer cases become very interesting to provide information on NPS prevalence. In this work, ten former cases of the National Institute of Toxicology and Forensic Science (INTCF) of Madrid Department (INTCFM), all of them belonging to defendants accused of crimes against public health and who had been found positive to ketamine, were reassessed. At the first toxicological analysis of those hair samples, a positive consume in ketamine had been determined by gas chromatography coupled to mass spectrometry (GC-MS). In this work, the same hair samples were reanalyzed by high-resolution mass spectrometry (UHPLC-HRMS/MS) using an incubation methanolic extraction combined with a single, simpler, non-selective and direct sample pre-treatment. After corroborating the GC-MS results previously obtained for the same samples, the detection of additional NPS using this new methodology evidenced its benefits and opened the possibility to perform a NPS prevalence study. In brief, in those cases with a positive consumption in ketamine, a polyconsumption of other drugs and NPS was found, including different

☆ #INTCF: National Institute of Toxicology and Forensic Sciences (Ministery of Justice). https://www.mjusticia.gob.es/es/ministerio/organismos-entidades/instituto-nacional

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arylcyclohexylamines as deschloroketamine, 3-MeO-PCP and methoxetamine; and cathinones as methylmetcathinone and N-ethyl-pentylone.

1. Introduction

Ketamine or 2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone, is an arylcyclohexylamine synthesized by Calvin Stevens in 1962, in the laboratory of Parke-Davis in Michigan [1,2] as an alternative analog of phencyclidine (PCP).

It began to be used as an anesthetic and analgesic and cataloged as a dissociative drug. It has been used in general anesthesia in humans and veterinary medicine [1,2] for the last fifty years.

As a curiosity, the dissociative effects of ketamine were described by the physician and scientist John C. Lylly after self-consumption, and he described its effects with the sentence "look across the border into other realities" [3]. Most likely, ketamine is illegally consumed due to its hallucinogenic and stimulants properties when consumed in high doses.

This substance and its analogs must be strongly monitored in Drug Facilitated Crimes (DFC) because ketamine analogs do not present color or smell and possess effects related to sedation, analgesia, immobility and amnesia [4,5].

In the world, this substance has experienced an increment in non-medical uses, with a high prevalence in Asia [6,7]. In Europe it appeared in 1990 mixed in illicit preparations altogether with stimulants (mainly MDMA) [8]. Because of the clinical use of ketamine, it was not included as a New Psychoactive Substance (NPS), but its combined use with NPS was established in the report on the risk assessment of ketamine in the framework of the joint action on new synthetic drugs of the European Monitoring Center for Drugs and Drugs Addiction (EMCDDA) [7]. Then, the International Narcotics Control Board (INCB), pursuant to resolutions 49/6, 50/3 and 57/10 of the Commission on Narcotic Drugs, called upon the governments to pay particular attention to the misuse and diversion of ketamine [7]. In Spain, ketamine was included as controlled substance in 2010 [9].

In toxicological and forensic cases, drugs are generally analyzed in different matrices (blood, urine, vitreous humor, hair, liver, gastric content, bile, kidney, lung, meconium, etc). The blood sample is the reference or sample of choice to quantify and interpret the concentrations of drugs and their metabolites of recent or acute intoxications. The hair samples present particularities and some additional advantages in different judicial investigations, such as a greater temporal window of detection, where the consumption in previous months or even years can be detected. In general, one centimeter of hair approximately reflects a period of one month (~1 cm/month) [10], where the proximal centimeters reflect the most recent consumption periods. The results are an average of the period analyzed, which is one of its limitations. The hair samples allow to investigate consumption patterns retrospectively and to know the polyconsumption pattern and prevalence of drugs and NPS consumed.

The systematic screening procedures for NPS analysis in hair are limited, and, in fact, the pretreatment procedures traditionally employed in targeted methods for hair analysis usually take much time and effort [11–14]. At present, ketamine is the most consumed arylcyclohexylamine associated to young consumers and polyconsumption profiles. There are around a hundred publications where its determination has been reported. These studies usually employ chromatographic separation techniques coupled to mass spectrometry [5,15–24]. Apart from the ketamine consumption, the research of other possible drugs or NPS is also very necessary from forensic cases with the aim of studying the prevalence of these substances in the population in a certain period. NPS are potentially dangerous and must be detected in forensic cases to reduce the damage in the society, as they may be sold on the Internet as "research chemicals" or "legal highs", accompanied by the indication that they are "not for human consumption". Analytically, NPS and their metabolites need to be detected with a highly sensitive and selective analytical technique. Traditional combined techniques such as gas chromatography coupled to mass spectrometry (GC-MS) usually do not present enough sensitivity and selectivity in the forensic analysis of NPS in hair. In addition, little information is known about NPS (mother drugs) and their metabolites in the human body. Generally, the mother drugs are present in the hair samples, which facilitates the identification, but the real forensic challenge comes when trying to detect the NPS metabolites in this matrix. These NPS and metabolites altogether will contribute to inform on the prevalence and detection of NPS in the population, or, at least, in subjects involved in criminal cases analyzed by certain forensic institutions. In this work, some Spanish criminal cases involving ketamine consumers studied in the INTCFM from 2017 to 2018 were studied under the hypothesis that this type of consumers might have a poly-consumption profile involving other psychoactive substances [11].

As a consequence, this work aims to report the analytical approach followed in the INTCFM to confirm the presence of drugs of abuse (ketamine and other drugs) and the identification of other potential NPS in hair matrices from ten real cases related to criminal actions occurred in Spain involving ketamine consumption. Those cases had previously given positive results in ketamine by the routine method GC-MS [11]. To achieve this goal, the specific objectives pursued in this work were: (i) identification of arylcyclohexylamines and their metabolites present in the analyzed hair samples by UHPLC-HRMS/MS; and (ii) study of prevalence considering all the substances identified, with special focus on NPS and other hallucinogens detected, involved in the polyconsumption profile of ketamine consumers. Firstly, the study included the comparison of instrumentation and analytical performance of a high-resolution mass spectrometry (UHPLC-HRMS/MS) method to the traditional GC-MS method. Since the high-resolution method uses a single simpler, non-selective and faster sample pre-treatment, the first step of the study sought to corroborate the GC-MS results previously obtained for the same samples. In a second step, the detection of other NPS and metabolites evidenced the benefits of using this new extraction and chromatographic-spectrometric method.

2. Experimental

2.1. Standards and reagents

LGC Promochem Cerilliant (Teddington, Middlesex, United Kingdom), Cayman Chemical (Ann Arbor, Michigan, USA) and Lipomed (Arlesheim, Switzerland) provided either pure solutions in methanol or acetonitrile at 1.0 mg/ mL or 1 mg (those which were solid) of the drug standards of several arylcyclohexylamines (ketamine, norketamine, phencyclidine (PCP), 3-methoxy-phencyclidine (3-MeO-PCP) and methoxetamine), two cathinones (N-ethylpentylone (ephylone) and 4-methylmetcathinone (mephedrone)), LSD, dextromethorphan, and other drugs standards: amphetamine-like compounds (amphetamine, 3,4-methylendioxy-N-methyl-amphetamine (MDMA or ecstasy), 3,4-methylendioxyamphetamine (MDA)

and 3,4-methylendioxy-N-ethyl-amphetamine (MDEA)). LGC Promochem Cerilliant also provided all the deuterated internal standards as 1.0 mg/ mL solutions in methanol: ketamine-d4, amphetamine-d5, MDMA-d5, methadone-d9, codeine-d3, morphine-d3, cocaine-d3 and benzoylecgonine-d3. All the stock solutions were stored at 4 ºC.

All the reagent solutions used for UHPLC-HRMS/MS sample pretreatment were of analytical grade (> 99.9%). Sigma-Aldrich (Saint Louis, Missouri, USA) provided the derivatizing substances, that were pentafluoropropionic anhydride (PFPA) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP).

2.2. Hair samples

The sampling of the hairs was performed following the sampling guidelines of the Society of Hair Testing (SoHT) [10]. Samples were: (i) blank hair of the same length (aliquot 0–3 cm), used to make the standard solutions for calibration and limit of detection (LOD) and limit of identification (LOI) from volunteers who had not taken any drugs or medication; and (ii) 10 hair samples collected from previous cases by the INTCFM. Table 1 summarizes the data of individuals and the characteristic of the lock of hair analyzed. All lock of hairs were subjected to a pretreatment stage.

In this work, we reanalyze the same length of hair that was analyzed in the initial routine by GC-MS, for a better and comparable evaluation of these cases by the laboratory.

3. Methods

Beforehand, a pretreatment method used and evaluated by the INTCFM was employed for the study of the hair samples using a simpler and universal procedure extraction to prepare them for a screening analysis.

3.1. Preanalytical hair sample treatment

The steps followed to pretreat the hair samples for GC-MS are described in detail in *Matey* et al. [11]. Briefly, the GC-MS sample preparation consisted in (i) decontamination with dichloromethane; (ii) trituration of the hair and specific extractions, with basic digestion (NaOH, 1 M) for amphetamines and ketamines; (iii) purification using liquid-liquid extraction (LLE) and solid-phase extraction (SPE); and (iv) derivatization using PFPA and ethyl acetate.

Another extraction process was performed for specific analysis of cocaine and opioids by GC-MS with methanolic incubation (T=60ºC and t=18 h), and similar process of (i, iii and iv). It is done when it is necessary to analyze these substances.

Table 1
Summary of the data of individuals (n=10) and hair analyzed.

N	Gender	Age (Years)	Total length (cm)	Analytical length (cm)	Color	Dyed (Y/N)
M-1	Male	-	4	4	Black	No
M-2	Male	25	3	3	Black	No
M-3	Male	32	5	5	Black	No
M-4	Male	-	5	5	Brown	No
M-5	Female	-	19	0–6[a]	Yellow/ brown	Yes
M-6	Male	-	22	0–6[a]	-	-
M-7	Male	24	33	0–6[a]	Black	No
M-8	Male	30	5	5	Black	No
M-9	Male	44	48	0–4[a]	Black	No
M-10	Male	24	33	0–6[a]	Black	No

[a] 0-X: The studied aliquot is from the hair root to the longest length of the analyzed locks of hair samples.

On the contrary, a single simpler sample treatment prior to the UHPLC-HRMS/MS analysis was made for all substances following the reference [25]. Briefly, it consisted in: (i) washing the hair samples twice with 5 mL of dichloromethane, and (ii) trituration of the hair and extraction of the analytes with methanol (methanolic incubation) for 18 h. Trituration was performed using a Precellys Tissue Homogenizer (from Bertin Instruments, Montigny-le-Bretonneux, France). All the internal standards were added prior to the methanolic incubation.

3.2. Instrumental methods

The GC-MS methodology was the one traditionally employed to characterize drugs and their metabolites in suspects' hair [11]. For UHPLC-HRMS/MS analysis of NPS, the pre-analytical extraction was a direct and non-selective (universal) extraction, adapted from Matey et al. [25,26]. The main analytical parameters for UHPLC-HRMS/MS analysis are summarized in Table 2.

In the case of the UHPLC-HRMS/MS method [25,26], the ionization mode was set up to electrospray ionization or H-ESI II in positive and negative ionization (switching), full scan ion monitoring using the same settings reported and described by A. G. Helfer et al. [27,28]. Phase A was added gradually to the Phase B, programmed as follows: 0–1.0 min 1% B, 1.0–10.0 min to 99% B, 10.0–11.5 min to 1% B and 11.5–13.5 min hold 1% B. The inclusion list for fragmentation was modified for different NPS and their corresponding metabolites molecular masses to be included and the full scan mode (FS).

After studying the analytical performance of the method in terms of selectivity and LODs and LOIs, every chromatogram and fragmentation MS spectrum obtained was compared to all the chromatograms and spectra stored up in the INTCFM library, which contains more than 1550 standards in its database, with a specific high-resolution MS/MS spectra [27,28].

3.3. Data treatments: LOD, LOI and selectivity calculations

To experimentally determine the limits of detection (LODs) and limits of identification (LOIs), decreasing concentrations (200, 50, 25, 20, 15, 10, 5, 2 and 1 pg/mg) spiked in hair blank samples were prepared with the same process of extraction of real hair samples. The LOD was defined as the lowest concentration exhibiting a MS signal, which was produced by the accurate precursor ion [28] and a chromatographic signal at least of 3-times the background height in the chromatogram [29–32]. The LOI was defined by two different criteria: (i) the accurate precursor ion must be detectable (LOD) [28] and (ii) the underlying HRMS/MS spectrum must contain at least the two principal fragments of the reference library spectrum [28–32] corresponding with the lowest concentration of the analyte in the hair matrix.

Table 2
Instrumentation and analytical parameters set for UHPLC-HRMS/MS. The extract was evaporated to dryness and reconstituted in 100 µL of methanol, from which only 1 µL was injected.

Parameters	UHPLC-HRMS/MS
Stationary phase	Phenylhexyl (100 mm×2.1 mm×2.6 µm).
Mobile phase	Phase A: ammonium formate 2 mM plus 0.1% formic acid (pH 3). Phase B: acetonitrile and methanol (50:50, v/v) plus 0.1% formic acid and 1% of Phase A. Gradient (increasingly adding the Phase B in A).
Detection	Orbitrap Q Exactive Focus System
Mode	Full Scan Ion Monitoring (FSI) with Data-Dependent Acquisition (DDA)
Injection	1 µL

The selectivity of the method was demonstrated by analyzing 10 different blank of hair matrices in aliquots of three centimeters, which had not detected these substances or ≤LOD.

4. Results and discussion

In this section, the analytical approach for high resolution mass spectrometry analysis of ten forensic cases belonging to defendants accused of crimes against public health and positive in ketamine are presented. The results confirmed the presence of drugs of abuse (ketamine and other drugs) and different NPS in hair matrices. Three subsections are described: (i) a comparison of the detectability by the traditional GC-MS and the UHPLC-HRMS/MS methods employed; (ii) the identification of arylcyclohexylamines and their metabolites present in the analyzed hair samples; and finally (iii) an approach to prevalence study considering the substances identified in the ten cases studied, focusing on NPS and hallucinogens involved in the polyconsumption of ketamine consumers.

4.1. Substance detectability in hair samples by UHPLC-HRMS/MS

Firstly, all substances detected previously by GC-MS [11], where it is also realized a estimate of the LODs and LOIs of standard substances by UHPLC-HRMS/MS method. Table 3 shows the substances detected by UHPLC-HRMS/MS and their corresponding experimental LODs [28] and LOIs [28–32]., The drugs, NPS and metabolites detected were ketamine, norketamine, MeO-PCP, methoxetamine, N-ethylpentylone (ephylone), 4-Methylmethcathinone (mephedrone or 4-MMC), 4-Bromo-2,5-dimethoxyphenethylamine (2C-B), dextromethorphan, fentanyl, amphetamine, methamphetamine, 3,4-methylendioxyamphetamine (MDA), 3,4-methylendioxymethamphetamine (MDMA), 3,4-methylendioxyethylamphetamine (MDEA). Another potential NPSs that were not detected were also analyzed just in case; such as different cathinones: 4-Methylethcathinone (4-MEC), 4-Methyl-alpha-pyrrolidinbuthiophenone (4-Methyl-PPP), alpha-pyrrolidinbuthiophenone (alpha-PPP), alpha-pyrrolidinpenthiophenone (alpha-PVP), methylendioxypyrrolidinovalerone (MDPV), Methylbenzodioxolylbutanamine (MBDB) Buphedrone, Pentedrone; Phenethylamines (PEAs): para-methoxyamphetamine (PMA), para-methoxymethamphetamine (PMMA), 25I-NBOME and 25C-NBOME; Synthetic cannabinoids (SC): JWH-018, JWH-073, JWH-122, JWH-210, AM-2201, UR-144 and XLR-11; Fentanyls: acetyl-fentanyl and cyclopropyl-fentanyl; Tryptamines: bufotenine and psylocine and Phenidates: ethylphenidate.

For comparison purposes, the LOD of ketamine, norketamine and methoxetamine and the LOD and LOI of 2C-B calculated in previous studies [25,26] have been also included in Table 3.

As expected, the calculated LODs and LOIs for the methanolic extraction followed by UHPLC-HRMS/MS methodology (Table 3) were lower than those values obtained for the conventional GC-MS methodology (preceded by a non-selective extraction). Lower LOD values imply better capability to detect smaller concentrations of analyte in the sample. In fact, ketamine had LOD values about two orders of magnitude lower with UHPLC-HRMS/MS than with GC-MS. Furthermore, different arylcyclohexylamines (deschloroketamine, methoxetamine [25] and MeO-PCP), cathinones (4-methylmetcathinone, N-ethylpentylone), hallucinogens 2C-B [26], DMT, LSD and opioids dextromethorphan were detected in these real samples using the UHPLC-HRMS/MS (preceded by a methanolic extraction).

Unlike the simple conventional GC-MS interpretation, the interpretation of all results from UHPLC-HRMS/MS requires some specific training. However, the higher detection capacity of UHPLC-HRMS/MS may facilitate the detection of occasional or single consumptions by untargeted approaches, as for example in Drug-Facilitated Crimes (DFC). To the greater number of identifications produced by a higher detection capacity of the UHPLC-HRMS/MS method, it was added the advantage of using a single method of analysis requiring half of the amount of sample (20 mg) in comparison to the routine and more laborious GC-MS method (requiring 40 mg).

Table 3
LODs and LOIs for new psychoactive substances (NPS) and some drugs and metabolites [available standard drugs spiked into hair (see Section 3.3)] detected by UHPLC-HRMS/MS. Substances are grouped according to EMCDDA classification.

Drug group detected (EMCDDA classification)[5]	Drugs, NPS and metabolites	LOD (pg/mg)	LOI (pg/mg)
Arylcyclohexylamines	Ketamine	2[25]	20
	Norketamine	2[25]	20
	Methoxetamine	5[25]	5
	MeO-PCP	5	200
	PCP	50	200
Cathinones	4-MMC	20	50
	4-MEC	50	50
	Buphedrone	50	50
	Pentedrone	50	50
	N-ethylpentylone	5	25
	4-Methyl-PPP	5	15
	Alpha-PPP	15	25
	Alpha-PVP	5	20
	MDPV	5	10
	MBDB	10	15
Phenethylamines (PEAs)	Amphetamine[a]	25	50
	Methamphetamine[a]	20	20
	MDA[a]	50	50
	MDMA[a]	20	20
	MDEA[a]	10	10
	4F-Amphetamine	20	50
	PMA	10	25
	PMMA	10	25
	2C-B	10[26]	50[26]
	25I-NBOME	5	10
Synthetic cannabinoids (SC)	JWH-018	25	25
	JWH-073	10	50
	JWH-122	20	50
	JWH-210	5	10
	JWH-250	15	25
	AM-2201	5	50
	UR-144	20	50
	XLR-11	5	10
Tryptamines	LSD[a]	5	15
	Bufotenine	25	50
	Psylocine	50	50
Opioids	Dextromethorphan[a]	2	10
	Fentanyl[a]	10	50
	Acetyl-Fentanyl	20	50
	Cyclopropyl-Fentanyl	15	50
Phenidates	Ethylphenidate	5	20

[a] Not classified as a New psychoactive substance, but it was also included due to its high consumption prevalence and high comorbidity associated.

4.2. Identification of arylcyclohexylamines and their metabolites in hair samples by UHPLC-HRMS/MS

Currently, the identification of arylcyclohexylamine substances and their main metabolites in real hair samples is relevant to assess the type of polyconsumption in ketamine consumers [11]. As mentioned above, through UHPLC-HRMS/MS, different arylcyclohexylamines were detected in these real samples as well as different metabolites that were not detected by the GC-MS method formerly. Some information about the metabolism of methoxetamine is described by Matey et al. [31]. In this study, another arylcyclohexylamine-type metabolite was detected alongside deschloroketamine and was tentatively proposed as dehydrodeschloroketamine based on the main HRMS/MS ion fragments present (see Supplementary material S1 and S2).

The results obtained in this work regarding the prevalence of arylcyclohexylamines in hair samples was similar to those found in non-biological samples with the data available at the INTCFM [33] in a similar period of time. Non-biological material seized analyzed in 2017 at the INTCFM (N=1991) contained arylcyclohexylamines (ACH)

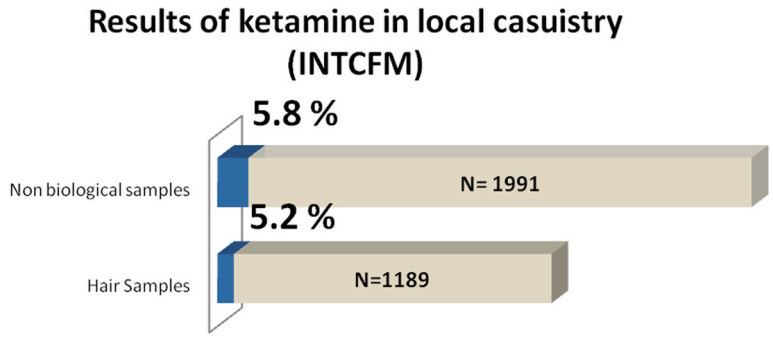

Fig. 1. Prevalence of ketamine in a similar period in hair and non-biological samples in the INTCFM. Hair samples were analyzed by GC-MS in the routine method [11] with n=62 positive cases for ketamine of a total seized analysis of N=1189 in 2017–2018 (15 months). Non-biological samples, were collected from our public memory of INTCF in 2017 with n=116 positive cases for ketamine in a total analyzed of N=1991 [33].

in 160 cases (8%), mainly ketamine (72.5% of the ACH cases, corresponding to the 5.8% of total samples analyzed in 2017 in INTCFM), deschloroketamine (21.3%), 3-MeO-PCP (2.5%), methoxetamine (2.5%) and deschloro-N-ethyl-ketamine (1.2%) [34].

Interestingly, similar results were obtained in hair during the period under study (N=1189, 5.2% of positive cases to ketamine) by GC-MS [11] (see Fig. 1). When hair samples were reanalyzed by UHPLC-HRMS/MS, the arylcyclohexylamines detected were in the same order of frequency than non-biological samples. Ketamine was detected in all the selected cases (N=10), deschloroketamine (2), methoxetamine (1) and 3-MeO-PCP (1), although the deschloro-N-ethyl-ketamine was not detected.

4.3. Substances identified in the selected forensic cases: drug prevalence

Results reported in [11] and our results showed in the previous section demonstrate the actual advantages for the detection of arylcyclohexylamines and their metabolites using the high-resolution technique of UHPLC-HRMS/MS. The additional information facilitates a prevalence approach focused on the drug abuse of NPS in ketamine consumers. The study of prevalence of NPS is interesting in hair matrices for several reasons: (i) most drugs are mainly not metabolized, and (ii) the accumulative consumptions during large periods can be detected along the hair, as reported by Fabresse et al. [13].

Therefore, the identification of NPS, their main metabolites and other drugs present in the hair samples of the ten cases studied confirmed, again, the polyconsumption profile of ketamine users. In the cases analyzed (N=10), again all drugs of abuse previously detected by the routine method by GC-MS, as well as new NPS that had not been previously tested using GC-MS, such as mephedrone, N-ethylpentylone (ephylone), and other three arylcyclohexylamines (methoxetamine, deschloroketamine and methoxy-PCP), they all were detected by UHPLC-HRMS/MS. These NPS were grouped as different arylcyclohexylamines and, besides them, two cathinones (methylmetcathinone (MMC) and N-ethylpentylone (ephylone)), and another interesting drug group such as hallucinogens, where three types of substances were detected: 4-bromo-2,5 -dimethoxyphenylethylamine (2C-B), tryptamines and dextromethorphan, which in high dose is known to have some hallucinogen effects [34]. These results are gathered in Fig. 2.

NPS methoxetamine, deschloroketamine and MeO-PCP were classified as arylcyclohexylamines (see Fig. 2, in red, four cases). Methylmetcathinone and ephylone were classified as cathinones (see Fig. 2, in blue, two cases). It must be highlighted that some of the hallucinogens present (Fig. 2 in green, six cases in total) may be also cataloged as NPS. LSD and DMT were detected and classified as tryptamines (two cases). Other hallucinogens are 2C-B (one case) and dextromethorphan (three cases), whose hallucinogenic effects are well-known when consumed in high doses [34].

In addition, other substances were detected: cocaine, benzoylecgonine, ethylbenzoylecgonine, norcocaine, tramadol, venlafaxine,

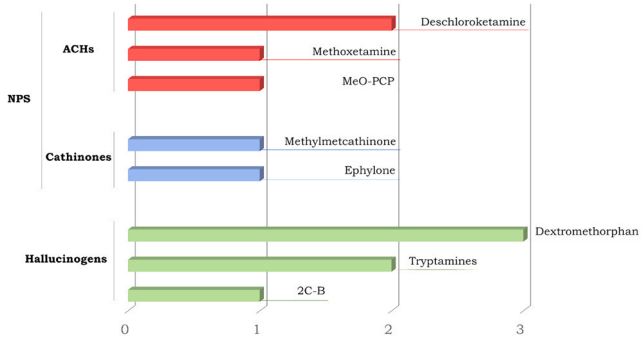

Fig. 2. Highlight about prevalence of some drugs detected in the forensic cases (N=10) involving a positive consumption of ketamine. X-axis gives the frequency of the referred drugs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

gabapentin, methylphenidate, haloperidol, aripiprazole, sildenafil, quetiapine, carbamazepine, olanzapine, levetiracetam, bromazepam, nordiazepam, diazepam, alprazolam and clonazepam. All this wide profile of substances and metabolites reflects a high polyconsumption behavior among ketamine consumers.

In a previous study of criminal forensic cases [11] where more than 1189 hair cases were analyzed by GC-MS, more than 5.2% had already been positive for ketamine. The consumer type was a young profile (median=29 years), polyconsumer, and probably for recreational experiences. The supplier is most likely in the darknet (illicit market on the Internet) [35].

The UHPLC-HRMS/MS method has proven better detectability than the traditional GC-MS method used in the INTCFM, obtaining lower limits of detection, up to two orders of magnitude lower.

5. Conclusions

Sample preparation by methanolic incubation is used in the laboratory of INTCFM prior to GC-MS only for specific target analytes. The extraction was improved, simplified and extended to every analyte obtaining a non-selective and universal process that requires less amount of sample (20 mg instead of 40 mg) for the subsequent analysis of Drugs, NPS and metabolites by high resolution mass spectrometric methods.

Regarding the prevalence or incidence of NPS considering ten forensic cases belonging to defendants accused of crimes against public health and with a positive consumption in ketamine, a polyconsumption of other drugs (hallucinogens and NPS) was also confirmed by. The concomitant consumption of classical drugs of abuse, stimulants and other drugs used for therapeutic or recreative consumption, reinforces the idea of the high polyconsumption behavior of consumers of ketamine.

CRediT authorship contribution statement

All co-authors: Conceptualization. **J.M. Matey, A. López-Fernández:** Experimental analysis. **All co-authors:** Investigation. **J.M. Matey, F. Zapata, A. López-Fernández:** Data analysis, Interpretation. **J.M. Matey:** Writing – original draft. **All co-authors:** Writing – review & editing. **G. Montalvo, C. García-Ruiz, M.A. Martínez:** Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of Interest

The authors declare that they haven´t conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2021.111080.

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